"On a Bacterial Disease of the Turnip (*Brassica napus*)." By M. C. Potter, M.A., F.L.S., Professor of Botany in the University of Durham College of Science, Newcastle-upon-Tyne. Communicated by Sir M. Foster, Sec. R.S. Received November 15,—Read December 6, 1900.

In the autumn, when the activity of the turnip plant is mainly devoted to the storage of reserve material and the characteristic roots are increasing in size, it is not uncommon in this neighbourhood to find among the plants still growing in the fields some whose roots are quite rotten and with a highly offensive and peculiar smell.

The plants thus affected can be recognised by the drooping, yellowish leaves, the older leaves being the first to show any indications of disease. They gradually flag and droop to the ground, at the same time becoming yellow and shrivelled in appearance. The leaves next in age gradually exhibit the same signs of premature decay, and this proceeds until finally the young leaves at the growing point succumb. The time taken for the collapse of the leaves naturally varies with different individuals, but it is usually about two weeks from the time of the first infection.

The roots of these plants when examined present a very characteristic appearance. The decaying portion may be of a greyish-white or dark-brown colour, and is quite soft to the touch; the cell wall has lost its natural firmness and the cells their turgidity, and with the escape of the cell-sap the tissues have been reduced to a soft watery pulp. In the particular disease now treated, the portion attacked remains of a whitish colour, and I have therefore described it under the name "White Rot," as my investigations have shown that this form of rottenness is due to a specific organism producing this particular colour when attacking a root. The brown and other discolorations found in similarly diseased roots are probably due in part to this organism, together with others, but I have not succeeded in cultivating the "Brown Rot," and this awaits further investigation.

The disease can be readily communicated to sound roots, it being sufficient merely to make a slight incision and smear a small portion of the rotten mass upon the injured surface for decay to be immediately set up. In twenty-four hours the previously healthy cells around the inoculated surface show the characteristic changes of form and colour to a depth of about a quarter of an inch, indicating the progression of the decay. Keeping the plant under observation, without further injury, it is noted that the rind bordering on the wound gradually becomes soft and assumes a lighter colour; the discoloration gradually extends; the older leaves, too, droop and change colour;

by degrees the entire rosette of leaves perishes, and the whole root becomes a soft, putrid mass, which eventually collapses, and after a shower of rain almost entirely disappears, exactly the same symptoms appearing as in the case of the plants found decaying in the fields. The most careful microscopic search has failed to detect any trace of hyphæ of the higher fungi in the decaying mass, but only a swarming mass of bacteria. The tissues are completely disorganised (see fig. 1), the cells separating from each other along the middle lamella, the cell-walls are soft, swollen, and faintly striated, the protoplasm too has lost its natural colour and become slightly brown and contracted, so that it no longer remains closely in contact with the cell-wall.

With a view to determine whether the bacteria are the cause of the rottenness, and if so, to isolate the particular organism which produces it, a series of cultures was undertaken.

In the first instance, a nutrient broth made from turnips was employed. Pieces of turnip finely chopped were steamed in a beaker until soft, sufficient tap-water being added to just cover them; when soft they were pressed through a cloth and the liquid filtered. To the clear light yellow filtrate thus obtained 5 per cent. of gelatine was added, and the mixture was then steamed, filtered, and drawn into test-tubes, which previously had been plugged with cotton-wool and exposed to a temperature of 140° C. for half an hour. These test-tubes, containing about 10 c.c. each of the bouillon, were next steamed for half an hour on three consecutive days, and as a further test of complete sterilisation they were incubated at 20° C. for a few days. No colonies were found to develop. (Whenever mention is made of test-tubes containing nutrient gelatine it must be understood that all have been prepared in this manner, and none have been employed which have not been submitted to these tests.) In some cases the broth was neutralised, in others it was allowed to retain the natural acidity of the cell-sap; but subsequently Koch's bouillon, neutralised with sodium hydrate by the phenolphthalein test, was found to give the most satisfactory results, and hence was always used.

In separating the various organisms found in the rotten mass a sterile platinum wire was introduced into the turnip (the rotten part practically offering no resistance), and then immersed in a test-tube (A) containing about 10 c.c. of the liquid nutrient gelatine. From this a loop was taken in a similar manner into a second test-tube (B), and so on until a sufficient degree of attenuation was reached. The test-tubes after being well shaken were turned out into petri capsules *a*, *b* . . . *g* respectively. These were placed in a cool incubator, and the colonies allowed to develop. In *a*, and often in *b*, the entire surface became covered with growing colonies too thickly crowded to be of any use for the purpose of isolation; but in the others the colonies were less numerous and sufficiently distinct to allow the organisms to be sepa-

rated from each other. The most conspicuous colonies were those which liquefied the gelatine; among others producing no liquefaction *Micrococcus candicans* and a yeast were especially noted for their frequent occurrence; but no trace of any of the higher fungi was found. The colonies were next transplanted by means of a sterile platinum wire into test-tubes containing about 10 c.c. of nutrient gelatine; and after numerous trials I was satisfied that pure cultures were obtained.

The various organisms as isolated were sown by means of a freshly-heated platinum wire upon sterile but living blocks of turnip. To prepare these blocks the turnips were first washed, and then soaked in a 1 per cent. solution of corrosive sublimate to destroy any organisms adhering to the outer surface, the corrosive sublimate being afterwards thoroughly washed away by means of water sterilised by discontinuous boiling. The rind was then removed by a sterile knife, the turnips being cut into suitable blocks on a sterile plate and quickly inserted in the test-tubes. Treated in this way the blocks of turnip, while quite sterile, were composed of healthy living cells, as was shown by three sets of control tubes. In the first set the blocks, prepared as above, were immersed in cooled liquid nutrient gelatine, in the second similar blocks were immersed in sterile water; in neither case were any colonies found to develop either when the blocks were partially or wholly submerged, and after eight days no sign of decay had appeared. In the third set the blocks were simply inserted in the tubes, and kept in a damp atmosphere; on microscopical examination cell division was observed to have taken place in the outer layers of uninjured cells, and the cell tissues presented a normal and entirely healthy appearance.

In the tubes containing the inoculated blocks many showed signs of advanced decay in about twelve hours, and all those in which any rottenness appeared were carefully noted.

After repeated experiment and a long series of cultures, I succeeded in isolating a bacterium which liquefies gelatine, and which, when sown on the sterile blocks of living turnip, produced the characteristic "White Rot" previously described.

The isolation of the bacterium in this manner was further confirmed by pricking out the colonies by means of Unna's harpoon. Small colonies of about 15 μ growing in a petri capsule were selected and transplanted by the harpoon into petri capsules containing some sterile turnip bouillon. A specially fine harpoon needle was obtained, but the point was still larger than these very small colonies, and it was only after some practice that they could be successfully transplanted. The colonies selected were those growing quite apart, which appeared to have arisen from a single bacterium, to eliminate as far as possible any chance of the needle touching more than one. Lest, however, even these small colonies might have grown from more than one bacterium,

a single bacterium was selected and its development watched with the capsule fixed under the microscope until the colony was sufficiently large to transplant. Cultivations were also made by the method of the hanging drop. A drop of gelatine bouillon from a test-tube containing a very few bacteria was placed upon a sterile coverslip, and then inverted over a sterile growing cell and examined under the microscope. If the bacteria were too numerous, the preparation was discarded and trials made until a hanging drop was secured with only one or two bacteria. The growing cell was now fixed under the microscope, so that a selected bacterium could be observed and the growth of the colony noted. When sufficiently large the coverslip was quickly inverted and the colony removed by the fine Unna's harpoon to a petri capsule. In this way pure cultures were obtained, grown from a single bacterium, which always gave rise to the characteristic "White Rot," and left no doubt that this bacterium is the sole organism concerned in the disease.

Pure cultures were also sown upon plants growing in the College garden with exactly the same result. The decay commenced at the point of infection and soon spread through the sound roots, eventually producing the same white putrefying mass of rottenness.

The bacterium can live for many generations as a saprophyte without losing its virulence as a parasite. A stock obtained from a "white-rotted" turnip growing in a field near Newcastle on September 10th, 1898, was isolated during that month, and after passing through several cultivations in successive test-tubes was finally put aside on April 29th, 1899. On August 23rd two sound turnips were selected in the College garden, and while still growing, the part of the roots above ground was washed with corrosive sublimate and afterwards with sterile water; a wound was then made with a sterile knife, and a little of the culture from one of the test-tubes left undisturbed since April 29th was introduced by a platinum wire. The turnips were then covered over with a zinc cylinder, and, upon examination five days after, on August 28th, the rot was found to have penetrated deeply into the tissues, the larger half of the roots having become completely rotten with all the distinctive characteristics of the true "White Rot."

In order to ascertain the precise action of the bacterium, and to determine whether it produced any ferment capable of acting upon the cell-wall in a manner similar to those of various parasitic fungi, the method of precipitation by alcohol was adopted. A litre flask was plugged, sterilised, and then filled about half-full with sterile blocks of turnips, to which was added a small block upon which a pure culture of bacterium had been sown; a little sterile water was then introduced, the flask closed as quickly as possible, and then well shaken to distribute the bacteria. In twenty-four hours many of the blocks showed the characteristic action of the bacterium, and in the

course of three or four days nearly the whole contents had become rotten.

The next important step was to separate the bacteria from their products. The contents of the flask were turned out and pressed through a cloth into a glass cylinder to remove the coarser portions, the turbid liquid was then filtered, and afterwards diluted with four to five times its bulk of alcohol. Almost immediately on addition of the alcohol a cloudy precipitate formed, and, at the end of twenty-four hours, a copious flocculent precipitate was deposited. After filtration the precipitate was washed with absolute alcohol, dried, carefully collected, and then digested with distilled water for about three hours. The solution was then passed through a Pasteur-Chamberland filter fixed in a Maassen's bacteria filter. In this manner a clear, pale, straw-coloured liquid was obtained free from bacteria. The liquid when drawn into sterile test-tubes remained clear for any length of time, but when exposed to the air it soon became turbid. A series of ten such sterile test-tubes was prepared, five of which were held over a Bunsen burner, and the fluid allowed to boil; the other five were left without any exposure to heat. Thin sections cut from sterile blocks of turnip, by means of a razor steeped in boiling water, were taken off in sterile water and quickly introduced both into the boiled and unboiled fluids. The action of the unboiled fluid was very marked. Fig. 1 shows a

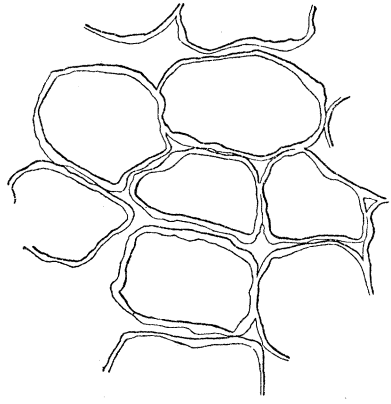

Fig. 1.—Group of cells from a section of turnip which has been exposed to the action of the cytase for twenty-four hours. The cell-walls are swollen and irregular in outline, and the cells are separating along the middle lamella (Zeiss, E. oc. 2).

section taken from one of these preparations after twenty-four hours' exposure: the cell-wall is swollen and striated, and so much softened that great difficulty was found in handling the section and removing it to the slide; it is well seen that the walls have quite lost their

natural firmness and clear regularity of outline, being bulgy and distended in places; the dissolution of the cells is very apparent along the middle lamella, and the whole appearance of the section corresponds exactly with those taken from turnips found affected by the rot in the fields. The sections contained in the boiled fluid exhibited none of the appearances described above, and the cell-walls remained perfectly normal. It is thus evident that the bacterium secretes an enzyme which dissolves the middle lamella and causes the softening and swelling of the cell-wall. Fig. 2 represents a single cell from a

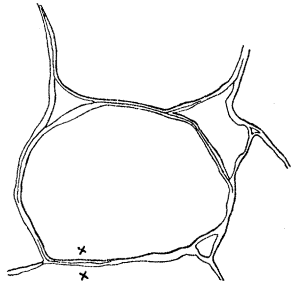

Fig. 2.—Cell immersed for sixteen hours in an unboiled solution of the cytase. Thickness of cell-wall, 2 μ at × × (Zeiss, E. oc. 2).

section immersed in the filtered, unboiled liquid for sixteen hours. Fig. 3 shows one after an immersion of forty hours. The thickness of

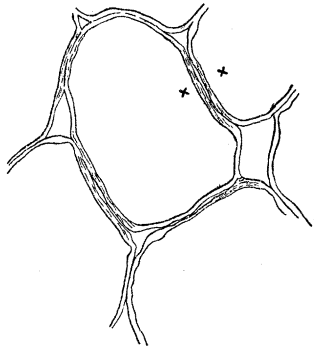

Fig. 3.—Section immersed for forty-two hours in unboiled solution of the cytase. Thickness of wall, 5·3 μ at × × (Zeiss, E. oc. 2).

the walls was 2 μ and 5·3 μ respectively. (I should remark here that these sections were cut out of season from old turnips in which the walls would be more resistant, and this would account for the rela-

tively slow development. In sections from more succulent growing roots the walls have been found to swell from 2 μ to 7 μ in the course of twenty-four hours.) In fig. 4 the cell is drawn from a section im-

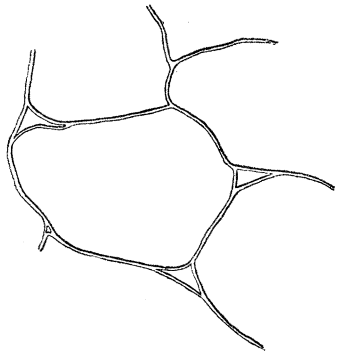

Fig. 4.—Section immersed in solution of cytase for forty-two hours, whose power had been destroyed by boiling. Cell-walls quite normal (Zeiss, E. oc. 2).

mersed for forty hours in the boiled liquid. The cell-wall is not perceptibly thickened or affected in any way.

The activity of the enzyme in the decaying plant was also shown by passing the juice from the bruised pulp directly through a Pasteur-Chamberland filter, when its action on the cell-wall was precisely that described in the case of the watery extract of the alcoholic precipitate.

The bacterium also secretes the enzyme when growing in a beef solution. Small flasks containing 100 c.c. of beef bouillon, inoculated with a pure culture, became turbid in the course of twenty-four to thirty-six hours. After an interval of eight days, the liquid was filtered and diluted with five times its bulk of alcohol, when a precipitate immediately began to appear. After standing twelve hours the precipitate was collected by filtration, dried, and then digested with 10 c.c. of distilled water. After filtration through a Pasteur-Chamberland filter, experiments were repeated as above with sections of sterile turnip, and the same results were obtained; the liquid was found to possess the property of dissolving the middle lamella, and causing the softening and swelling of the cell-wall. All action of the ferment was destroyed by boiling.

To avoid the tedious process of the filtration through a Pasteur-Chamberland filter, and the necessary sterilisation of the apparatus, various attempts were made to render the solutions aseptic by the use of such re-agents as chloroform, thymol, formalin, &c. But this process had to be abandoned, as in all these cases living bacteria were found after twenty-four hours, and no reliance could be placed upon it.

In the early stages of the investigations, filtration—except when

the elimination of bacteria was desired—was effected through ordinary filter paper. The quantity of the precipitate being small, the portion of the paper upon which it was deposited was cut out and digested with water. But in order to avoid any possible action of the enzyme upon the paper, kieselguhr was subsequently invariably employed, a few pieces of glass at the base of the funnel covered with a little asbestos serving to prevent the kieselguhr from passing through, the necessary pressure to ensure filtration being derived from an air-pump.

The filtered extract from the rotten turnip also contains a diastasic ferment. Two test-tubes, each containing 5 c.c. of the dissolved ferment, were diluted with 5 c.c. of a 1 per cent. starch emulsion, one of the test-tubes having previously been boiled. After twenty-four hours the test-tube with the unboiled ferment showed no starch reaction on the addition of iodine; but the boiled tube at once gave the characteristic blue.

Similar diastasic enzymes are excreted by several other bacteria (Lafar).

It will be convenient here to say that, adopting Migula's classification, I have ventured to name the bacterium I am describing *Pseudomonas destructans*, though the description will be given later.

It has been established that *P. destructans*, both when living in a nutrient solution and on a living turnip, excretes an enzyme which has the power of dissolving the middle lamella and of causing the softening and swelling of the cell-wall.

As a further result of the bacterial action, as already described, the protoplasm of the cells is found to have contracted, become brown, and separated from the cell-wall, showing evidence of the action of a toxin secreted by the bacterium. The same effect was produced in living turnip cells when treated with the boiled pressed juice of a turnip, which had become rotten through the influence of a pure culture of *P. destructans*. The pressed juice was filtered and about 10 c.c. drawn into test-tubes, which were then plugged and sterilised by discontinuous boiling. Sections cut by a razor, sterilised by boiling, from blocks of sterile, living turnip (p. 444) were quickly transferred to the boiled juice after it had cooled; at the same time similar sections were immersed in test-tubes containing the same quantity of sterile water. After twelve hours a very marked contrast was observable between these sections. In those immersed in the sterile water the cells presented the normal appearance, with the protoplasm pressed close to the cell-wall, while in those in the boiled pressed juice the protoplasm was dead, had assumed a brown tint, and contracted away from the cell-wall. A toxin, therefore, which is not destroyed by boiling is secreted by *P. destructans*.

In his paper "Ueber einige Sclerotinien und Sclerotien-Krank-

heiten," de Bary has shown that oxalic acid is secreted by the hyphæ of *Peziza sclerotiorum* when living as a parasite, and that this acid acts as a toxin in killing and plasmolysing the protoplasm. Wehmer has found that *Aspergillus niger* and *Penicillium glaucum* also form oxalic acid when growing in a sugar-containing solution. Oxalic acid, being an unavoidable product in the metabolism of the higher plants, and also in some fungi, it seemed reasonable to suppose that it might be found as a similar product in the life of bacteria. With this idea I tested the juice expressed from a rotten turnip, and found, on addition of calcium chloride, a precipitate which proved to be calcium oxalate. Cultures were then undertaken to test for the presence of oxalic acid as a product from *P. destructans*. A broth, made by steaming small pieces of actively growing turnips until soft, was neutralised by an excess of calcium carbonate and filtered; it was then allowed to stand overnight, when a further deposition of calcium took place; it was then again filtered, clarified with the white of an egg, steamed, filtered, and drawn into four flasks, each containing 150 c.c., which were sterilised. A solution was thus obtained free from any oxalic acid which might have been present in the tissues of the turnip. Two of the flasks were inoculated with *P. destructans* on August 28th. In twenty-four hours they became turbid, and after four days were tested and found to contain oxalic acid; while the control flasks showed no evidence of this acid, and remained perfectly clear.

P. destructans also sets up an oxalic fermentation in Pasteur's solution. A litre of Pasteur's solution with cane sugar was made up and divided into four flasks, each of which was carefully sterilised and one sown with *P. destructans*. After twenty-four hours the liquid in the inoculated flask, which was previously perfectly clear, became cloudy, and after a week quite opaque; 10 c.c. of this, when treated with a solution of calcium chloride, in the presence of acetic acid, at once showed a precipitate of calcium oxalate, which increased on being warmed. Another 10 c.c. of the original solution, which had been kept sterile during the same period, remained quite clear on treatment with the same re-agents. *P. destructans* thus sets up an oxalic fermentation in a sugar containing liquid. It has also been found that carbon di-oxide is given off during the process.

When treated with alcohol, the Pasteur solution, in which *P. destructans* had been growing for eight days, yielded a white flocculent precipitate which contained the cytase. The oxalic acid, however, remained in solution, and was deposited as the calcium salt on addition of calcium chloride. This calcium precipitate, when mixed with manganese di-oxide and treated with sulphuric acid, yielded carbonic acid, which furnishes a further confirmatory test of the presence of oxalic acid. The precipitation by alcohol affords a ready method of separating the toxin (oxalic acid) from the cytase, and this explains

why the sections treated with the watery extract of the alcoholic precipitate exhibited no marked plasmolysis.

That the oxalic acid formed by *P. destructans* in Pasteur's solution acts as a powerful toxin was very clearly shown. Six plugged and sterile test-tubes were prepared, and about 10 c.c. of the fermenting Pasteur solution was poured into each. To three of these (series 1) sufficient calcium carbonate was added to neutralise the oxalic acid. Both series of tubes were then sterilised by discontinuous boiling, during which process the cytase would be destroyed, and into both when cool freshly cut and sterile sections of turnip were placed prepared as described on page 446, and the solutions allowed to act till next morning. The sections in the second series of test-tubes showed a marked contraction of the protoplasm, and it looked brown and dead, and showed no tendency to return to its normal condition when immersed in pure water. In the first, which were treated with calcium carbonate, the protoplasm was quite normal, and exactly resembled a section which had been immersed in sterile water for the same period.

A third set of test-tubes were filled with about 10 c.c. of the solution; these were not boiled, and received no calcium carbonate; the sections introduced showed complete dissociation of the cells, the cell-walls greatly swollen and the protoplasm very strongly contracted. This experiment with the Pasteur solution demonstrated the production of the same cytase, and strikingly illustrated its effect upon the plant cell, as well as the toxic action of the oxalic acid; even more so than was the case with the same experiment with turnip juice.

In considering the effect of the oxalic acid upon the cells, it is important to note that calcium pectate, a salt which is decomposed by oxalic acid with the production of calcium oxalate, enters largely into the composition of the middle lamella. Wehmer has shown that in the cultivation of *Aspergillus niger* and *Penicillium glaucum* oxalic acid is formed in saccharine solutions, that the oxalic acid produced acts as a toxin to these fungi, and gradually diminishes their vigour, and that when a certain strength has accumulated no further development is possible; growth, however, is resumed when the oxalic acid is neutralised by a calcium salt. The reaction between the oxalic acid produced by *P. destructans* and the calcium pectate of the middle lamella is precisely analogous: the oxalic acid would be neutralised, and the pectate replaced by the oxalate, and the continued growth of the bacteria would thus be rendered possible. The oxalic acid* then both acts as a toxin in killing the cells and may also play some part in

* Since the above account of the formation of oxalic acid by *P. destructans* was written, Zopf has published a note also describing the formation of oxalic acid by *B. xylinum*, "Oxalsäurebildung durch Bacterien," 'Berichte d. D. Bot. Gesell.,' Feb. 1900.

the destruction of the middle lamella and the separation of the cells.

Fig. 5 shows a cell swarming with *P. destructans*; the bacteria are seen occupying the inter-cellular spaces and lying in the track of the middle lamella.

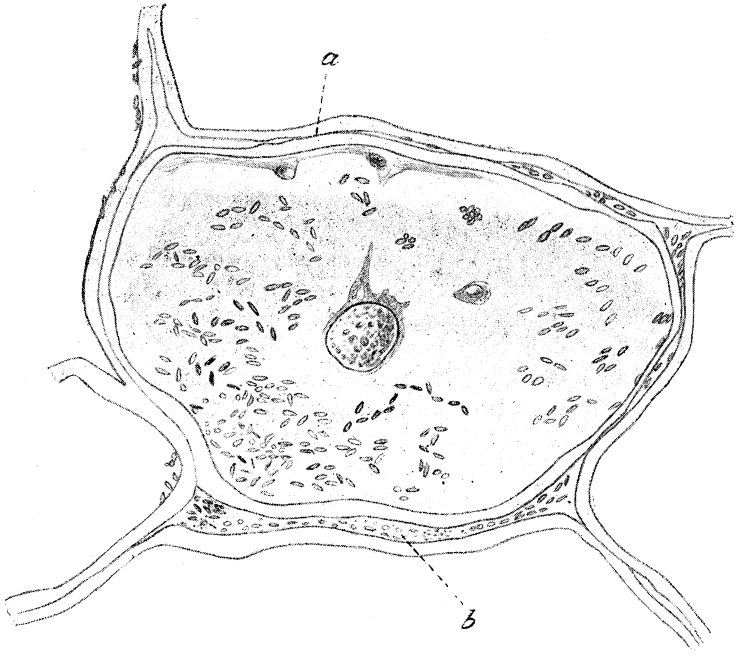

Fig. 5.—A cell from turnip inoculated with a pure culture of *P. destructans*. The bacteria are seen in the cell cavity and also along the track of the middle lamella, and in the intercellular spaces. The cell-wall is much swollen; at *a* it is just beginning to separate along the middle lamella and at *b* the dissociation is more strongly marked. The nucleus and portions of the protoplasm still remain. (Drawn with Abbe camera lucida, Zeiss, E. oc. 4.)

In the case of several parasite fungi, the hyphæ also burrow in the thickness of the cell-wall, and the same phenomenon is now shown to be true of one parasitic schizomycete, and possibly this is owing to the necessity for the neutralisation of the oxalic acid as a condition of existence.

Enzymes similar in nature to that described for *P. destructans* have been demonstrated by Marshall Ward for *Botrytis* and by de Bary for *Sclerotinia*.

The action of this bacterium upon living plant tissues is precisely similar to that of certain of the parasitic fungi; in both cases the invading

organism produces oxalic acid, which acts as a toxin to the protoplasm and, decomposing the calcium pectate, furthers the dissolution of the cells; and also there is the secretion of a cytase, which has a destructive action upon the cell-wall and intercellular substance. The question of the parasitism of the bacteria thus stands in these respects on the same platform as that of the fungi, and a complete homology is established between them.

At first I experienced considerable difficulty in staining the flagella. Loeffler's method was first tried, but with no positive results; it enabled one, however, to notice two deeply stained portions at either end of the rod. Van Ermengen's method also failed in spite of the strictest attention to technique, but by gradually increasing the strength of the silver nitrate, and finally using a 2 per cent. solution, the desired result was obtained, and the bacillus was then found to possess *one polar flagellum* (fig. 6). It should be mentioned that the practice of passing the cover-slip thorough a flame was discarded in favour of drying the cover-slip at 60° C. in a water bath, the latter method being more certain and giving better results.

Fig. 6.—*Pseudomonas destructans* with single polar flagellum. (Swift's 1/12th apochromatic and compensating ocular 12.)

Pseudomonas is aerobic. A stab culture rapidly develops along the track of the wire, forming a funnel; the edge of the funnel reaches the sides of the test-tube in about forty-eight hours, and gradually sinks as the gelatine becomes liquid. The gelatine, however, is never wholly liquefied, the liquefaction extending down the sides of the tube only to a depth of about one and a half centimetres. If a layer of gelatine is immediately poured above the stab and the test-tube placed in the incubator, the track of the wire is clearly marked out as before, but the colonies soon cease to develop, and all growth ceases after three days. The tube may be kept for many weeks in this condition.

Again, so far as my experiments show, the action of *Pseudomonas* upon turnips and potatoes only takes place in the presence of oxygen. The following are typical examples of experiments frequently repeated and always with the same results:—A flask holding about 250 c.c., with a tightly fitting indiarubber cork perforated to admit two glass tubes bent at right angles, was sterilised in the following manner. The tubes were plugged at each end with cotton wool, and the plugs pushed well into the tubes, the flask being also plugged with cotton wool, and, together with the glass tubes, sterilised by dry heat. Mean-

while the indiarubber cork was boiled for half an hour in a 10 per cent. solution of corrosive sublimate. The flask having cooled was then about half-filled with sterile blocks of living turnip, prepared as described above, and inoculated with a pure culture of *P. destructans*. The indiarubber cork, after being washed in sterile water, was quickly inserted into the flask, the glass tube being pushed through the perforations and the junctions sealed with melted wax. The longer tube (A) reached to the bottom of the flask, the shorter (B) only slightly protruded downwards through the cork. The action of the bacterium could be detected in the course of twelve hours, the blocks changing colour and showing signs of disintegration at the edges. During fermentation, a considerable quantity of gas was given off, which could be collected from B over a pneumatic trough, the fluid which soon accumulated at the bottom of the flask rising in A, supplying the requisite pressure. When the longer tube A was left open, and a sufficient supply of oxygen could diffuse into the flask, carbonic acid was continually given off, and in the course of about a week the contents became entirely rotten and reduced to a watery mess. When, however, in a precisely similar flask used as a control, the longer tube after a short interval was closed, and the shorter connected with a tube for collecting any gas given off, thus cutting off the supply of oxygen, the evolution of CO_2 soon ceased, and, as far as could be observed, the action of *P. destructans* ceased also.

To carry this point a step further, and to ascertain more definitely whether the action of *Pseudomonas* could take place in the absence of oxygen, another series of flasks was fitted up with the two tubes as already described, the same precautions as to sterilisation being adopted, and the prepared blocks of turnip introduced and inoculated as before. The shorter tube was now connected with a second flask containing an alkaline solution of pyrogallic acid, and the other with a bent tube containing mercury to act as a manometer, and prevent any access of oxygen from the air. The first result noticed was an expansion of the air in the flasks, the mercury rising in the distal limb. The mercury continued to rise, bubbles of carbon dioxide eventually escaping round the bend. This action, however, ceased in the course of two days, the available supply of oxygen in the flasks and intercellular spaces being exhausted. After a long interval (four months—June 6 to October 5) the flasks were disconnected, and the turnip blocks examined. They still retained their original shape, and were only rotten superficially; the pieces had somewhat lost their rigidity, but offered considerable resistance when stretched. Microscopic examination showed all the cells to be dead, but it was only one or two layers of superficial cells which showed any evidence of bacterial action. The cell-walls on the outside of the block were swollen and striated, and could be readily separated along the middle lamella; the

cell-walls in the interior of the tissue, however, presented the normal appearance, neither swollen nor readily separating.

Control experiments were set up, in which, after four days, the manometer and pyrogallic flask were disconnected, and the air allowed to diffuse into the flasks; upon subsequent examination the blocks in these were found to have become completely rotten. We may thus infer that the action of *P. destructans* only proceeds so far as a supply of oxygen is available.

Potatoes as well as turnips were employed in these experiments, and the results in each case were the same, except that with the potato when the flask was connected with the pyrogallic flask and manometer, immediately after the inoculation of the blocks, no bubbles of CO_2 were observed to escape round the bend, and there was no indication of the rot.

Characters of Pseudomonas Destructans.

Habit.—On growing turnips producing a "White Rot" in the living tissues.

Morphology.—Short motile rods, $3\mu \times 8\mu$, with a single polar flagellum.

Cultures can only be made in the presence of oxygen.

Gelatine.—Petri Capsules. Forms circular colonies of whitish-grey liquefying gelatine.

Stab Cultures. Grows rapidly along the track of the wire, forming a funnel-shaped tube of liquid gelatine, with a white, cloudy deposit in the liquid portion.

Agar.—White, glazy growth.

Turnips.—Grows rapidly as a parasite.

Potato and Carrot.—Same effect as on the turnip.

Beetroot.—No growth as a parasite.

Broth.—Koch's bouillon and turnip; becomes cloudy and opaque.

Ferments.—A cytase, causing the swelling and softening of the cell-wall, and dissolution of the middle lamella.

A diastase. A peptonising ferment, producing liquefaction of gelatine.

Toxin.—Oxalic acid formed as a product of metabolism in turnip-juice and in Pasteur's solution containing cane sugar.

Stains.—Readily stained with the ordinary aniline dyes, but not with Gram's method.

Reaction.—Residual product always acid.

Copious evolution of carbonic acid during the fermentation.

Among various bacteria at present noted as causing plant diseases, that described by Kramer as attacking the potato (Nassfäule) approaches most nearly to the one which is the subject of this paper. Kramer's

bacillus agrees in liquefying gelatine very rapidly, and it destroys the middle lamella, and finally the cell-wall. The size of the bacterium as given by Kramer is from 2·5 μ long, and 7–8 μ broad, very nearly the same dimensions as those of the turnip bacterium. Kramer, however, has not named his bacillus, and he makes no mention of the flagellum. He describes two stages in the decay of the potatoes. First, an acid stage, during which butyric acid and carbonic acid are given off; in this stage the sugars, then the intercellular substance, and finally the cell-walls are destroyed: the starch is not attacked. Subsequently, the proteids are broken up with the formation of ammonia, methylamine, trimethylamine, and other products; in this stage the acids are neutralised. In the action of *P. destructans* upon turnips and potatoes carbonic acid is given off, and the reaction of the pulp is always acid. On referring to a chemical friend, he could not definitely state that butyric acid, methylamine, and trimethylamine are also produced; he was of opinion that they were present, but that the decomposition is of a more complicated nature. *P. destructans* differs from Kramer's bacillus in secreting a diastase, and always yielding an acid product; further, *P. destructans* liquefies the gelatine in circular areas, the leaf-like formation described by Kramer never having been observed, nor have I ever found the apparently unjointed threads as much as 16 μ long upon nutrient plates. Pammel and Smith have also described a *Pseudomonas* (*P. campestris*), which causes a "brown rot" in the root and leaves of various cruciferous plants, evidently quite a distinct form.

The action of the bacteria upon the cell-wall of the higher plants has been studied by several observers. Van Tieghem, probably working with mixed cultures, has ascribed the destruction of cellulose to *Bacillus amylobacter*. Van Senus has isolated an enzyme and demonstrated its solvent power upon cellulose, from two bacteria, one anaerobic, living symbiotically. Winogradsky and Fribes have isolated an anaerobic bacterium which dissolves the middle lamella in the process of "flax-retting," and sets free the bast fibres, without, however, having any action upon the cellulose. Arthur ascribes the action of bacteria in the bacteriosis of carnations to an enzyme, but without isolating it.

Till quite recently I was unaware that any one had isolated from the bacteria an enzyme capable of attacking the middle lamella of living cells, and thus causing a plant disease. Laurent's valuable paper, "Recherches Expérimentales sur les Maladies des Plantes," I only obtained in August of this year. It was published in December, 1898, simultaneously with a preliminary paper I read at the University of Durham Philosophical Society; but previously, as early as January, 1898, I made a brief report to the Royal Society embodying the results of my work, viz., the isolation of the specific bacterium causing the

"White Rot" of turnips, and the isolation of an enzyme which dissolved the middle lamella and caused softening and swelling of the cell-wall. The pressure of teaching has prevented my publishing the complete paper sooner.

Laurent, in his investigations upon the potato and the causes of its greater or less resistance to bacterial disease, also established the existence of a cytase, which dissolved the middle lamella, rapidly softened the cell tissues, and caused the disaggregation of the cells.

The organism which was the chief subject of Laurent's researches, *B. coli communis*, is very rarely capable of living as a parasite upon potato-tubers and other plants. He states that it was necessary for the tubers to be deprived of resistance, by means of exceptional cultures, to enable the bacillus to develop upon the potato. From that point its virulence was increased by successive cultivations upon tubers of slight resistance, until varieties at first highly resistant ended by becoming invaded by the parasite. The virulence disappeared as soon as the microbe ceased to be cultivated on a living tuber, cultures in nutritive solutions sufficed to suppress the aptitude of the parasite, and henceforward it could only be restored after special preparation in alkaline solutions.

P. destructans, on the contrary, flourished on nutritive media and even after many cultivations could readily be inoculated from these on to pieces of living turnip, producing all the effects of the rot in about twelve hours; cultures both on nutritive media and on the turnip also rapidly invaded the tissue of the potato. Whether, therefore, it has any existence in a saprophitic form or not, it has evidently become strongly established as a parasite attacking the turnip, and probably is not confined to the turnip alone.

Wehmer has recently attempted to show that bacteria are not parasitic in the case of the wet rot (Nassfäule) of the potato, and that their action is only secondary. He maintains that bacteria only attack dead or unhealthy tissue, that the warmth and moisture of the damp chambers impair the health of the cells, and infection is only possible under conditions which renders the tissues morbid. The wet rot, Wehmer says, begins with a maceration of the tissues; between the separating dead cells numerous small bubbles are to be seen and masses of a small rod-like schizomycete. The initial stage is one of pectin-fermentation, succeeded by cellulose fermentation. With these processes are associated two special forms of bacteria. Wehmer's description of the rotting tissues agrees with my own, but he makes no mention of the enzyme nor of cultures of the bacteria. His conclusions that bacteria are not parasitic cannot be accepted in view of the isolation of the special enzyme by Laurent and myself, and of my experiments proving the infection of sound, healthy turnips when growing under perfectly natural conditions.

From numerous observations in the fields, I have come to the conclusion that *P. destructans* is always introduced at a wounded surface. Except in cases in which the decay has proceeded to a large extent, the point from which the decay spreads is always indicated by a wound in the epidermis and subjacent tissues. This observation is supported by the failure to infect sound roots except by first making a small incision, and from numerous trials it would appear that *P. destructans* is powerless to set up decay unless placed in contact with the parenchyma-cells of the cortex. Wounds caused by various snails, slugs, and larvæ, by which the bacterium could gain an entrance, are frequently to be seen on the roots, and I have no doubt the bacteria gain an easy entrance by this means. That slugs can and do carry the various disease-producing organisms has been shown by Smith in the case of the cabbage brown rot by *Agriolimax agrestis* and the larvæ of *Plusia brassicæ*, and of the tomato brown rot by the larvæ of the Colorado beetle. G. Wagner's experiments also conclusively prove that the spores of various parasitic fungi are very commonly distributed by snails.

Bacterial disease of turnips is much more common than is generally recognised, and the one now described is often very destructive to the crops, not only in the field but in store during the winter. On examining numerous specimens sent me for investigation, I speedily found that what is generally known as "finger and toe" or "grub," is by no means confined to *Plasmodiophora brassicæ*, but that many other organisms, either singly or in combination, play a very important part in the destruction of living turnips and swedes. Finger and toe is everywhere so prevalent that in considering the nature of turnip attack it is often too hastily assumed that *Pl. brassicæ* is the sole cause of the disease, and that the other effects are merely secondary. In addition to bacteria and *Pl. brassicæ*, I have found the turnip and swede crops to be attacked by *Fusarium* and also by *Botrytis*, and it is probable these do not exhaust the list of vegetable parasites for this crop, but further research is necessary before it is possible to separate the various organisms and assign to each its *rôle*.

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"The Micro-organism of Distemper in the Dog, and the Production of a Distemper Vaccine." By S. Monckton Copeman, M.A., M.D., F.R.C.P. Communicated by Sir M. Foster, Sec. R.S. Received November 14,—Read December 6, 1900.

(From the Brown Institution.)

Distemper is so fatal a disease of dogs, more particularly of such as are highly bred, that a method of preventing invasion by the disease has always been a desideratum.

As the result of investigations into the bacteriology of this disease, carried out in continuance of those commenced in my laboratory at St. Thomas's Hospital about ten years ago by the late Everett Millais, I find that the specific micro-organism concerned is a small cocco-bacillus, which stains with the ordinary aniline dyes, but is decolorised by the method of Gram. It grows readily on the surface of agar at body temperature; the individual colonies when isolated by the method of plate-culture having a greyish, glistening, semi-translucent appearance by reflected light, and a light-brownish tint by transmitted light. The general form is circular, but occasionally, and specially in primary growths, the edge is somewhat irregular. The microbe also grows well in beef-broth, causing at first a general turbidity. Later on, a deposit falls to the bottom of the tube, and the supernatant liquid becomes somewhat clearer. In cover-glass preparations from broth cultures the bacilli are not unfrequently found united together to form chains, sometimes of considerable length. The bacillus is capable of growing,

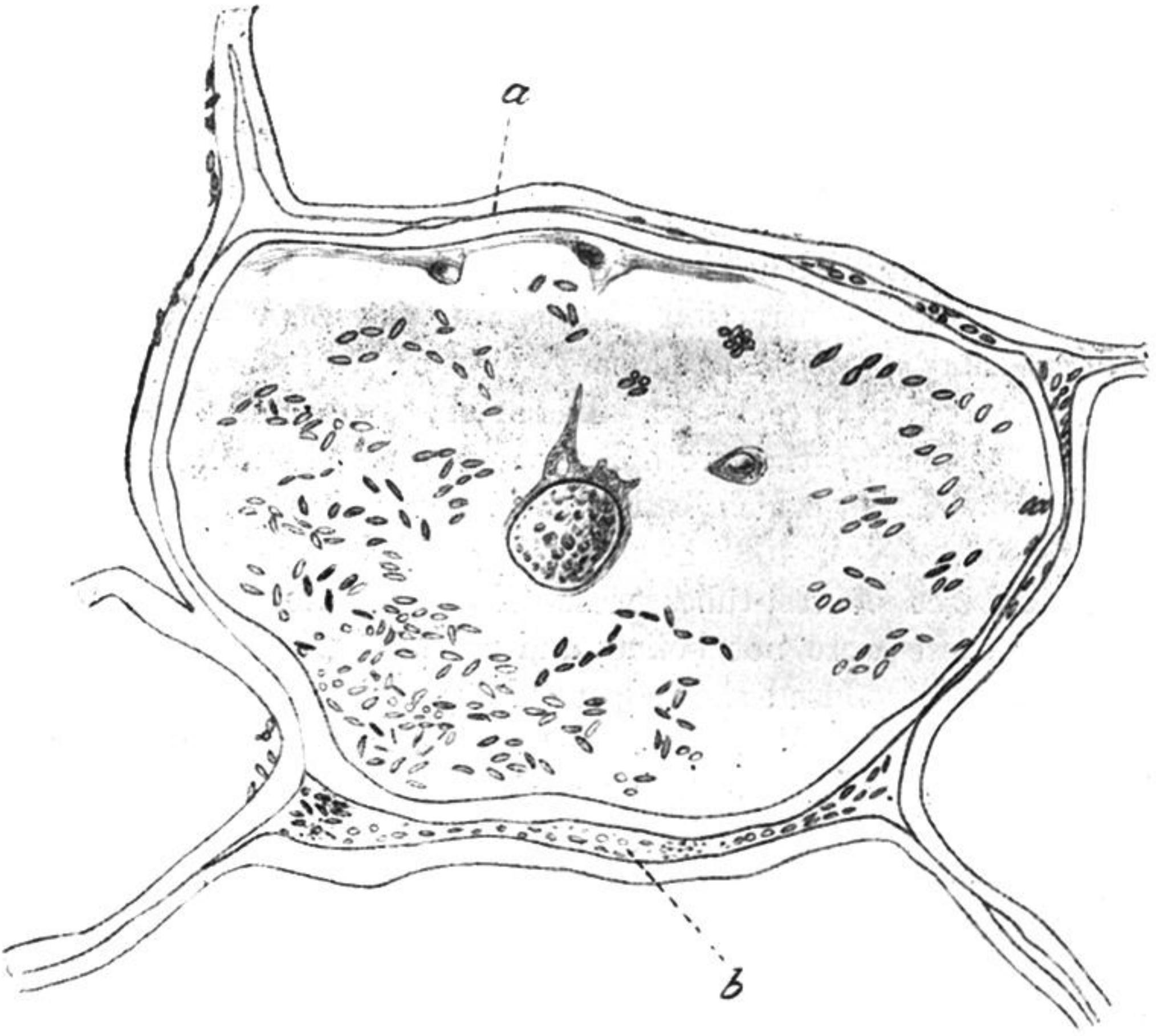

Fig. 5.—A cell from turnip inoculated with a pure culture of *P. destructans*. The bacteria are seen in the cell cavity and also along the track of the middle lamella, and in the intercellular spaces. The cell-wall is much swollen ; at *a* it is just beginning to separate along the middle lamella and at *b* the dissociation is more strongly marked. The nucleus and portions of the protoplasm still remain. (Drawn with Abbe camera lucida, Zeiss, E. oc. 4.)